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REVIEW

Potassium channels in gastrointestinal smooth muscle

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Summary

1 Electromechanical coupling in smooth muscle serves to coordinate the contractile activity of the syncytium. Electrical activity of smooth muscle of the gut is generated by ionic conductances that regulate and in turn are regulated by the membrane potential of smooth muscle cells. This activity determines the extent of Ca^{2+} entry into smooth muscle cells, and thus, the timing and intensity of contractions.

2 Potassium channels play an important role in regulating the excitability of the syncytium. The different types of K^+ channel are characterized by different sensitivities to membrane potential, to intracellular Ca^{2+} levels and to modulation by agonists.

3 This review highlights the different types of K^+ channels found in gut smooth muscle and describes their possible roles in regulating the electrical activity of the muscle.

Introduction

The gastrointestinal (GI) tract is surrounded by smooth muscle that generates the forces necessary for the mixing and processing of food and for propulsion of digestive matter along the gut. Generally speaking, smooth muscle cells in the gut are arranged into bundles that form two orthogonally apposed layers: the inner circular muscle layer (CM) and the outer longitudinal muscle layer (LM). The strength of contractions generated by these layers is largely determined by the amplitude, duration and frequency of action potentials, which mediate rapid influx of Ca^{2+} into smooth muscle cells and subsequent activation of the contractile machinery. Potassium (K^+) channels actively participate in shaping the electrical activity of smooth muscle by taking advantage of the large trans-membrane gradient in K^+ concentration to generate outward currents (or outward flow of positively charged ions). These currents are produced because the concentration of K^+ inside the cell (~ 140 mM) is higher than in the extracellular fluid (2–5 mM), causing K^+ to flow out of the cell through K^+-selective channels. Therefore, driving the membrane potential of cells further negative if the cell is at its resting potential and towards the equilibrium potential for K^+ (about -90 mV). The opening of K^+ channels is associated with restoration of the resting potential and inhibition of contractile activity.

The diversity in the types of K^+ channels found in smooth muscle of the gut reflects the fine tuning they exercise over the electrical activity of the syncytium. This syncytium is formed by smooth muscle cells connected to each other both anatomically and electrically through gap junctions which allow current to flow intracellularly from one cell to the other, thus enabling the mechanical activity of the constituent smooth muscle cells to be coordinated. In this review I will attempt to summarize recent and past data from patch-clamp studies, microelectrode studies and contraction studies that have addressed the role of K^+ channels in GI smooth muscle and describe their role in controlling its excitability.

Voltage-gated K^+ (K_v) channels in GI smooth muscle

General properties

All GI smooth muscles studied express voltage-gated potassium (K_v) channels. Typically, K_v channels are minimally activated at the resting membrane potential (RMP) but make a small but finite contribution to the overall resting K^+ conductance (g_K). K_v channels increase their probability of opening (P_o) with membrane depolarization

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and P_o usually peaks (approaches unity) at potentials positive of 0 mV. The voltage-dependence of P_o manifests itself under voltage-clamp conditions as a progressive decrease in the latency between the onset of a depolarizing step and opening of the channel, with increasing levels of depolarization (see Pongs, 1992). Thus, whole-cell K_v channel currents (I_{K_v}) which represent the summed activity of thousands of K_v channels in the cell membrane activate faster with increasing depolarization.

With maintained depolarization, K_v channels have a tendency to 'inactivate' and to cease conducting current. The rate of inactivation of K_v channels is described by one or more exponential functions and varies from one channel type to another. The time constant(s) of inactivation, which range from milliseconds to many seconds, are often used to distinguish one type of K_v channel from another (Table 1). Although the inactivated state and closed state are both non-conducting states, inactivated channels may only re-open after the membrane potential is brought back to a negative voltage (near the RMP), to allow the inactivated channels to 'recover'. During recovery, which may take milliseconds to hundreds of milliseconds, inactivated channels move back into the closed state from which they can re-open upon membrane depolarization. Inactivation consists of at least two separable processes (Dolly & Parcej, 1996). Rapid 'N-type' inactivation occurs over tens of milliseconds and involves block of the internal mouth of K_v channels by a cytoplasmic 'ball' peptide when the channel is open (Hoshi, Zagotta & Aldrich, 1991). Recovery from inactivation occurs when the 'ball' peptide dissociates from the mouth of the channel, following repolarization of the membrane potential. The slower 'C-type' inactivation involves the block of the outer mouth of K_v channels by conformational changes in the C-terminal region of the channel protein.

Based on their rates of activation and inactivation, two types of I_{K_v} can be distinguished in GI smooth muscles: (i) $I_{K_{vfast}}$ which peaks in < 10 ms at 0 mV; and (ii) $I_{K_{vslow}}$ which has a time-to-peak (TTP) of > 50 ms (see Table 1). In general, a rapid rate of activation is associated with a rapid rate of inactivation (Table 1); $I_{K_{vfast}}$ (at 0 mV) in most smooth muscles inactivates with time constants of 50–100 ms whereas $I_{K_{vslow}}$ inactivates more slowly over several seconds. Another important difference between $I_{K_{vfast}}$ and $I_{K_{vslow}}$ is that $I_{K_{vfast}}$ activates at potentials some 10–20 mV more negative than $I_{K_{vslow}}$ (see Table 1) which suggests that I_{vfast} channels influence the subthreshold electrical activity of the muscle.

Single-channel conductances

The single-channel or unitary conductance (γ) is a measure of how rapidly ions pass through a channel when it is in a conducting (open) state. Unitary conductance represents an 'electrical fingerprint' of sorts but precise measurement of γ is often difficult because of the small amplitude (< 1 pA) of the single-channel currents that are generated by K_v channels. In smooth muscle cells of the CM of the guinea-pig colon, the γ of K_{vfast} channels was estimated to be 13 pS under a symmetrical high-K^+ concentrations across the membrane (Vogalis & Lang, 1994). These channels opened briefly within the first 10–20 ms after a step depolarization and were largely inactivated at potentials positive of -30 mV. The rate of inactivation of the ensemble-averaged current closely matched the inactivation rate of the whole-cell $I_{K_{vfast}}$ recorded from the same cells (Vogalis, Lang, Bywater & Taylor, 1993). Similarly fast inactivating K_{vfast} channels with unitary conductances < 20 pS are also present in smooth muscle cells isolated from the mouse ileum (Fig. 1) and also in smooth muscle cells of the opossum oesophagus which also expresses a prominent $I_{K_{vfast}}$ (Akbarali, Hatakeyama, Wang & Goyal, 1995).

Little is known about the single-channel properties of $I_{K_{vslow}}$ channels in GI smooth muscle. In the CM layer of the canine colon, the K^+ channels responsible for the 4-AP-sensitive portion of the whole-cell $I_{K_{vslow}}$ have a conductance of ~ 20 pS in symmetrical high-K^+ solutions (Koh, Sanders & Carl, 1996) and inactivate with a similar time course as the whole-cell current (Thornbury, Ward & Sanders, 1992b). Two other voltage-gated channels with conductances of 90 and 150 pS have also been recorded from these cells (Koh *et al.*, 1996). Opening of the 90- and 150-pS

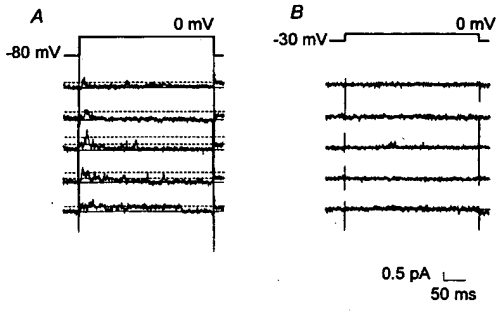

Figure 1 Cell-attached patch recordings from a smooth muscle cell of the mouse ileum showing activation of K_{vfast} channels upon depolarization of the patch from -80 to 0 mV (panel A). These channels were not available for activation from a holding potential of -30 mV (panel B). Solid lines through traces indicate closed channel level and dashed lines indicate open channel levels. TEA (5 mM) was added to the pipette solution to block BK channels in the patch. Cells were bathed in high-K^+ physiological solution and the pipette was filled with normal physiological solution (5.4 mM K^+) (F. Vogalis, unpublished observations).

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Table 1 Properties of K_v channels in GI smooth muscle

	$I_{K_{vfast}}$						$I_{K_{vslow}}$					
Tissue	V_{act} (mV)	$V_{0.5\ in}$ (mV)	τ_{in} (ms)	τ_{rec} (ms)	4-AP (mM)	TEA (mM)	V_{act} (mV)	$V_{0.5\ in}$ (mV)	τ_{in} (s)	4-AP (mM)	TEA (mM)	Ref.
Oesoph. oposs.	−50	−57	50	125	<3	~200	−30	–	s	>3	<10	(2)
Oesoph. cat	−60	–	–	–	–	–	–	–	–	–	–	(11)
Colon rabbit	−50	−50	~50	–	–	>20	−20	–	s	–	–	(3)
Colon rat	−40	−52		63	0.3	>10	−20	−55	s	>3	4	(17)
Ileum rat LM	−50	−71	~30	46	2	~90	−20	–	–	>5	~6	(12)
Colon g.p.	−60	–	–	–	<3	~130	−30	–	–	>5	<10	(15)
Taenia g.p.							−10	−51	–	–	–	(18)
Ileum g.p.							−30	–	s	–	4.8	(6)
Mouse ileum	−50	–	~50	–	<3	>2	−30	–	s	>3	<10	(8)
Antrum dog							−30	–	s	–	~5	(10)
Pylorus dog							−40	–	s	–		(16)
Colon dog							−40	−36	s	0.25	5	(13)
Colon dog	−30	–	s		0.07	>10	−30	–	s	>10	2.6	(4)
Colon dog							−30	−50	s	–	1.5	(5)
Colon dog LM							−40	−63	s	~12	~5	(14)
Oesoph. rab. MM							−20	–	s	>4	~5	(1)
Colon cat							−40	–	s	–	<20	(3)
Ileum rab. LM							−30	–	s	–	4.8	(9)
Ileum g.p. LM							−30	–	–	–	–	(7)

V_{act}, most negative membrane potential at which outward current is detectable in response to step depolarization of the cell; $V_{0.5\ in}$, membrane potential at which the maximum amplitude of the current that can be activated by a standard step depolarization, is halved.

Reference key: 1, Akbarali (1993); 2, Akbarali *et al.* (1995); 3, Bielefeld, Hume & Krier (1990); 4, Carl (1995); 5, Cole & Sanders (1989); 6, Duridanova & Boev (1995); 7, McHugh & Beech (1995); 8, Molleman, Thuneberg & Huizinga (1993); 9, Nagasaki, Komori, Tamaki & Ohashi (1993); 10, Sims (1992); 11, Sims *et al.* (1990); 12, Smirnov, Zholos & Shuba (1992); 13, Thornbury, Ward & Sanders (1992a); 14, Thornbury, Ward & Sanders (1992b); 15, Vogalis *et al.* (1993); 16, Vogalis & Sanders (1991); 17, Xiong, Sperelakis, Noffsinger & Fenoglio-Preiser (1995); 18, Yamamoto, Hu & Kao (1989).

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channels was reduced after removal of Ca^{2+} from the cytoplasmic surface of excised patches suggesting that they may be partially Ca^{2+}-dependent. This property is similar to a 40-pS K_v channel expressed in smooth muscle cells of the mouse ileum which is also stimulated by internal Ca^{2+} and also undergoes inactivation (Vogalis, Zhang & Goyal, 1998b).

Pharmacology of K_v channels

Typically, delayed rectifier-type currents such as $I_{K_{vslow}}$ are decreased in amplitude by >50% by tetraethylammonium (TEA) applied to the external surface of cells at concentrations of 5–10 mM. However, $I_{K_{vfast}}$ is much more resistant to external TEA (see Table 1) and in guinea-pig colonic myocytes equimolar substitution of external Na^+ (135 mM) with TEA only reduced $I_{K_{vfast}}$ by ~30%, while 5 mM of 4-aminopyridine (4-AP) also applied externally completely blocked this current (Vogalis *et al.*, 1993). Similarly in canine colonic CM cells, 4-AP blocks a rapidly activating I_{K_v} ($I_{K_{dr}}$; Carl, 1995) while block of the slowly developing component of I_{K_v} requires high (>10 mM) concentrations of TEA. Molecular biological studies on cloned K_v channels have shown that the sensitivity of K^+ channels to TEA is determined by the presence of amino acids with aromatic side-chains (tyrosine or phenylalanine) situated near the external mouth of the channel protein (Heginbotham & Mackinnon, 1992). The binding site for the membrane-permeable 4-AP on the other hand is situated near the internal mouth of K_v channels (see Pongs, 1992). TEA also has a separate internal binding site, a feature which has been exploited in colonic smooth muscle to help separate different components of the whole cell I_{K_v} (Carl, 1995).

Although peptide toxins have been shown to block certain native K_v channels in non-smooth muscle cells as well as cloned K_v channels (Chandy & Gutman, 1995), their actions on GI smooth muscle K_v channels have not been studied widely. In smooth muscle cells of the mouse ileum, however, the 40-pS K_v channel is sensitive to both charybdotoxin (ChTX) and to apamin (Vogalis *et al.*, 1998b). This same channel may be expressed in smooth muscle cells of the human colon in which apamin has been reported to suppress a voltage-sensitive non-inactivating K^+ channel current which is dependent on Ca^{2+} influx (Duridanova, Gagov, Dimitrov & Boev, 1997).

Function of K_v channels

In smooth muscle cells isolated from the circular muscle layer of the guinea-pig proximal colon, activation of $I_{K_{vfast}}$ can be detected at potentials just positive to the resting potential of intact strips

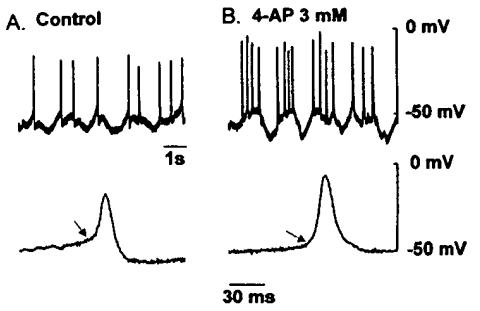

Figure 2 Increase in the excitability of circular muscle of guinea-pig colon by 4-AP. A, Activity recorded with microelectrode in the absence of 4-AP. Lower panel shows an action potential with slow depolarizing phase at the arrow. B, In the presence of 4-AP, the excitability is increased and the action potentials appear to lack the slow depolarizing phase (arrow, lower panel) (F. Vogalis, unpublished recordings).

of circular muscle (about −50 mV) (Vogalis *et al.*, 1993). This suggests that K_{vfast} channels may participate in setting the resting potential of this muscle, despite their tendency to undergo rapid inactivation at depolarized potentials. This is because, although $K_{v_{fast}}$ channels spend most of their time in the closed state, at potentials near the RMP, they continuously cycle between the inactivated, closed and open states. Therefore, the brief and sporadic openings of thousands of $K_{v_{fast}}$ channels may be sufficient to produce a small but persistent outward current that contributes to the resting potential of the cell. Intracellular recordings from strips of CM from the guinea-pig colon have shown that 4-AP increases the firing rate of smooth muscle cells and abolishes the slowly rising pre-potential that precedes the upstroke of the action potential under normal conditions (Fig. 2), allowing action potentials to rise more rapidly and peak at more depolarized potentials. This suggests that the role of $K_{v_{fast}}$ channels may be to dampen the rate of depolarization, and increase the threshold for action potential firing. The effect of this will be to decrease the availability of voltage-gated Ca^{2+} channels, which also undergo voltage-dependent inactivation, and thus lead to a decrease in Ca^{2+} entry. This mechanism may be important for maintaining the CM layer in a relatively quiescent state and suggests that suppression of $K_{v_{fast}}$ channels by excitatory transmitters may be a mechanism for increasing the electrical excitability and contractile activity of GI smooth muscle.

Although only a small fraction of of $K_{v_{fast}}$ channels (<10%) are available for activation at membrane potentials around −50 to −60 mV (Vogalis *et al.*, 1993), the relatively high input resistance (10^9 ohms) of colonic smooth muscle cells means that opening of only a small number

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of $K_{v_{fast}}$ channels may be sufficient to influence the excitability of smooth muscle cells. For example, given a unitary conductance of 13 pS, a single $K_{v_{fast}}$ channel would generate a current of 0.06 pA under a physiological K^+ gradient at −50 mV. If the open probability of a channel at −50 were 0.1 and there were 2000 channels per cell of which 200 were available to open (see Vogalis *et al.*, 1993), then the outward current generated by $K_{v_{fast}}$ channels would be $20 \times 0.06 = 1.2$ pA. This amount of current would produce a 6 mV hyperpolarization in a cell with an input resistance of 5×10^9 ohm.

In strips of canine pyloric CM, TEA (5–10 mM) increases the frequency and amplitude of action potentials and depolarizes the muscle by 5–10 mV (Sanders & Vogalis, 1989). This suggests that in pyloric muscle, TEA-sensitive K_v channels may contribute to the resting potential. TEA, however, also blocks large-conductance Ca^{2+}-dependent K^+ (BK) channels (see below) at much lower concentrations (Carl, Lee & Sanders, 1996) suggesting that the actions of TEA on muscle strips may not be attributable entirely to block of K_v channels.

In general, the LM layer in the GI tract has a less negative RMP than the CM layer and is more likely to generate spike discharges, e.g. in the canine pylorus (Vogalis & Sanders, 1990) and canine colon (Thornbury, Ward & Sanders, 1992a). This difference in excitability may be attributable in part to the differential expression of specific types of K_v channels. For example, the I_{K_v} in smooth muscle cells of the LM layer of the canine colon is relatively insensitive to 4-AP (Thornbury *et al.*, 1992a), whereas the I_{K_v} in smooth muscle cells from the CM layer is almost completely blocked by 1 mM 4-AP (Thornbury, Ward & Sanders, 1992b). Similarly, in the guinea-pig proximal colon, the 4-AP sensitive $I_{K_{vfast}}$ which is found in cells of the CM layer (Vogalis *et al.*, 1993) is absent in cells of the LM layer (F. Vogalis, unpublished observation).

A further physiological role for $I_{K_{vfast}}$ channels in smooth muscle may be to electrically tune the excitability of the cell membrane by actively filtering out fast rapid depolarizing transients. For example, if a wave of depolarizing current spreads to the cell from a another region of the tissue and begins to change the membrane potential at a rate which is slower than the rate of inactivation of $I_{K_{vfast}}$ channels in the cell membrane (50–100 ms), then the resulting depolarization will spread across the cell to neighbouring cells with minimal attenuation of its amplitude (determined by the passive electrical properties of the smooth muscle syncytium). However, if the rate of depolarization induced by the incoming signal has a rate of rise faster than the inactivation rate of $I_{K_{vfast}}$ channels, then this will result in the recruitment of more and more $I_{K_{vfast}}$ channels to the open state. This results in the activation of an outward current that opposes further depolarization. In the intestine, this mechanism may be important in limiting the propagation of spikes from the LM back to the CM, and preventing muscle spasm. In addition, active filtering of fast depolarizing transients may ensure that the smooth muscle in the CM contracts in a coordinated manner and only does so when a sufficiently large area of the syncytium becomes excited synchronously, also helping to prevent muscle spasm. This mechanism may be particularly important in tubular viscera such as the intestine to ensure that contractions occur as annuli, thus enabling the intestine to propel its contents efficiently.

Molecular identity of K_v channels

Molecular biological studies over the past decade have identified key features of the basic structure of K_v channels that are responsible for the their diverse pharmacological and functional properties (Chandy & Gutman, 1995). However, assignment of the various cloned K_v channels to their native counterparts has been problematic because not all the properties of cloned channels match all those of native channels. A complicating factor is that the cellular origin of the template mRNA from which channels are cloned is not known because the source tissue, for example GI smooth muscle tissue, contains a variety of cell types including nerves, vascular smooth muscle cells and endothelial cells. In addition, some of the discrepancies between the properties of cloned and native K_v channels may arise because native channels consist of large complexes of different protein subunits, some of which modify the properties of the main pore-forming subunit (Rettig *et al.*, 1994). Finally, the expression system itself (e.g. *Xenopus* oocytes) may confer properties on the cloned K_v channels that are normally not found in native channels.

The majority of cloned K_v channels are classified according to the degree to which their primary stuctures resemble the four basic groups of K_v channels encoded by four different genes in *Drosophila* (see Chandy & Gutman, 1995). The K_v1 family of channels encoded by the *Shaker* gene is probably the largest. Variants of this family, namely $K_v1.2$ and $K_v1.5$, have been cloned from smooth muscle tissue derived from the CM layer of the canine colon (Hart *et al.*, 1993; Overturf *et al.*, 1994). Northern blot analysis has revealed that mRNA encoding these channels is also present in smooth muscle tissue from other regions in the GI tract and mRNA encoding $K_v1.5$ is also present in vascular smooth muscle (Overturf *et al.*, 1994). $K_v1.5$ channels expressed in oocytes are pharmacologically similar to native $K_{v_{slow}}$ channels in canine colonic CM in that they are both sensitive to TEA and resistant to ChTX. It is also possible that native $K_{v_{slow}}$ channels may consist of a mixture of $K_v1.2$ and $K_v1.5$ subunits. This is because the pharma-

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cological and kinetic properties of heteromultimeric $K_v1.2/K_v1.5$ channels, expressed in oocytes by coinjection of cRNAs encoding $K_v1.2$ and $K_v1.5$, more closely resemble the properties of the native $I_{K_{vslow}}$ than the corresponding current produced by either clone expressed on its own (Russell *et al.*, 1994). More recently, an 18-pS K_v channel of the K_v2 family was also cloned from individually collected smooth muscle cells from the CM of the canine colon (Schmalz *et al.*, 1998). When expressed in *Xenopus* oocytes, these $K_v2.2$ channels generated a current that was moderately sensitive to both TEA and to 4-AP, thus fitting the pharmacological profile of native ~20-pS K_v channels found in canine colonic CM which is responsible for $I_{K_{vslow}}$ (Carl, 1995).

Candidates for the molecular equivalent of the rapidly inactivating $K_{v_{fast}}$ channels in GI smooth muscle include $K_v1.4$- and K_v4-like channels (Pongs, 1992). $K_v1.4$ channels are more sensitive to 4-AP than $K_{v_{fast}}$ channels and have much slower recovery rates (hundreds of milliseconds vs. minutes). Rapidly inactivating K_v4-like channels, however, which are TEA-resistant and 4-AP-sensitive (Chandy & Gutman, 1995) have similar recovery rates as $K_{v_{fast}}$ channels. Recently, mRNA encoding $K_v4.2$ was detected in smooth muscle derived from the guinea-pig colon (Ohya *et al.*, 1997). Smooth muscle cells from this tissue expresses a prominent $I_{K_{vfast}}$ (Vogalis *et al.*, 1993). Some members of the K_v3 or *Shaw* family also show rapid inactivation (Chandy & Gutman, 1995) but these channels (e.g. $K_v3.4$; Weiser *et al.*, 1994) are all blocked by low (2 mM) concentrations of TEA (Chandy & Gutman, 1995), thus ruling them out as potential candidates as the $K_{v_{fast}}$ channel homologues.

Modulation of K_v channels by second messengers

The primary structures of cloned K_v channels point to numerous consensus sequences for phosphorylation by serine/threonine and tyrosine kinases (see Chandy & Gutman, 1995). It is unclear, however, whether phosphorylation of these sites alters channel function or whether phosphorylation/dephosphorylation of these sites is associated with post-translational modifications of the channel proteins. In smooth muscle cells of canine colon, cAMP-dependent protein kinase (PKA) and agents such as VIP that increase cAMP production have been shown to increase the open probability of 4-AP-sensitive K_v channels in cell-attached patches (Koh *et al.*, 1996). These same channels are responsible for the membrane hyperpolarization elicited by agents that stimulate cAMP production (Du, Carl, Smith, Sanders & Keef, 1994). In the guinea-pig colonic CM, however, isoprenaline, a β-adrenoceptor agonist, and a membrane-permeable analogue of cAMP were both found to inhibit $I_{K_{vfast}}$ under whole-cell conditions (Vogalis *et al.*, 1998a). Suppression of $I_{K_{vslow}}$ can also be elicited by muscarinic receptor stimulation in guinea-pig colonic myocytes, which maybe account for the increase in spike activity produced by cholinergic receptor stimulation in colonic smooth muscle. Muscarinic receptor stimulation also suppresses cloned $K_v1.2$ and $K_v1.5$ channels expressed in oocytes through a Ca^{2+}-dependent PKC-mediated pathway (Vogalis, Ward & Horowitz, 1995). This suggests that suppression of K_v channels by G-protein-coupled receptor stimulation may be a mechanism for producing sustained increases in cell excitability and increased intestinal motility.

Nitric oxide (NO), a putative inhibitory transmitter in the gut, has also been shown to stimulate the opening of 80-pS K_v channels in cells of the canine colonic CM (Koh, Campbell, Carl & Sanders, 1995). These effects are mediated in large part by the direct actions of NO on the channels and can be inhibited by pretreating cells with reducing agents. These channels may participate in the NO-mediated inhibitory junction potentials (IJPs) recorded in canine colonic smooth muscle.

Arachidonic acid (AA) which is generated by the action of cytoplasmic PLA_2 on membrane phospholipids has been shown to suppress $K_{v_{fast}}$ channels in smooth muscle cells of the guinea-pig ileum (Nagano, Imaizumi & Watanabe, 1997). In toad gastric myocytes, however, AA and other negatively charged fatty acids derived from AA activate a stretch activated K^+ channel (Ordway, Walsh & Singer, 1989; Petrou *et al.*, 1995) suggesting that K_v channels are directly modulated by membrane lipids whose effects are species-specific.

Ca^{2+}-dependent K^+ channels

Heterogeneity of Ca^{2+}-dependent K^+ channels

In most types of smooth muscle cells, a concurrent rise in intracellular Ca^{2+} with depolarization, is accompanied by a large outward K^+ current. A large portion of this outward current is generated by the opening of BK_{Ca} channels which have unit conductances of between 250–300 pS and are found in all GI smooth muscles (Benham, Bolton, Lang & Takewaki, 1986; Carl *et al.*, 1996; Mitra & Morad, 1985; Singer & Walsh, 1987). BK channels behave much like K_v channels in that they are activated by voltage (depolarization) but the threshold depolarization at which they begin to open is shifted to more negative potentials as $[Ca_i^{2+}]$ increases from 100 nM to the submicromolar range. This dual dependence on membrane depolarization and cytoplasmic $[Ca^{2+}]$ causes BK channels to become active at the peak of Ca^{2+}-mediated action potentials in GI smooth muscle and to contribute to the repolarization phase of the action potential.

Apart from BK channels, there now appears to be several other types of K^+ channels that contribute

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to the overall Ca^{2+}-dependent K^+ conductance of GI smooth muscle. These channels include the intermediate conductance 90- and 150-pS K^+ (IK) channels in myocytes of the CM of the canine colon (Koh *et al.*, 1996), the 10- and 40-pS K^+ channels in mouse ileal smooth muscle cells (Vogalis & Goyal, 1997; Vogalis *et al.*, 1998b)and the 5-pS small conductance (SK) channels in mouse colonic smooth muscle cells (Koh, Dick & Sanders, 1997). The gating and pharmacological properties of these SK channels differ markedly from BK channels.

Functional properties of BK channels

The molecular identification of BK channels in canine colonic CM smooth muscle has revealed many structural similarities with cloned K_v channels (see Vogalis *et al.*, 1996). Molecular biological studies have shown that the Ca^{2+} sensitivity of BK channels resides in a C-terminal region (the 'Ca^{2+} bowl') which is unique to BK channels (see Schreiber & Salkoff, 1997). BK channels cloned from canine colonic smooth muscle (c-*slo*) show alternative splicing in this C-terminal region suggesting that different isoforms of BK channels with different Ca^{2+} sensitivites may be expressed in the same tissue (Vogalis *et al.*, 1996).

The Ca^{2+} sensitivity of cloned BK channels is enhanced significantly by co-expression of an accessory (β) subunit (Vogalis *et al.*, 1996). The Ca^{2+} sensitivity of multimeric channels (composed of α and β subunits) approaches that of native BK channels which typically require ~1 μM Ca_i^{2+} to open at potentials negative of 0 mV (see Carl *et al.*, 1996). Given that the bulk cytoplasmic [Ca^{2+}] in smooth muscle at rest is ~100 nM, it seems unlikely that BK channels contribute significantly to the resting potential in GI smooth muscle. This conclusion is supported by microelectrode recordings from canine colonic CM which show that ChTX, a potent blocker of BK channels, has no effect on the resting potential (Carl, Bayguinov, Shuttleworth, Ward & Sanders, 1995). Similarly, in the CM layer of the guinea-pig stomach, ChTX had little or no effect on the resting potential but increased the amplitude of action potentials triggered by slow wave depolarizations (Suzuki *et al.*, 1993) consistent with BK channels activating predominantly at depolarized potentials following rapid Ca^{2+} entry. In LM of the canine colon, however, ChTX was shown to depolarize the muscle and to increase spiking activity suggesting that BK channels in this tissue may be activated at the resting potential (Carl *et al.*, 1995). Similar results were reported in the LM of the guinea-pig ileum in which ChTX depolarized the muscle and increased action potential discharges (Hong, Roan & Chang, 1997). All these findings suggest that BK channels in the CM cells may have different Ca^{2+}- or voltage-sensitivities from those in LM, although a recent study in the opossum oesophagus reported that no such differences were detectable (Hurley, Preiksaitis & Sims, 1999).

In intact cells, BK channels tend to open in bursts, indicating that the concerted opening of many BK channels almost simultaneously gives rise to spontaneous transient outward currents (STOCs) which have been recorded from smooth muscle cells from many GI smooth muscles (Benham & Bolton, 1986; Bolton & Imaizumi, 1996). STOCs are believed to be activated by pulsatile release of Ca^{2+} from ryanodine-sensitive Ca^{2+} stores, triggered by Ca^{2+} influx through L-type Ca_v channels (Bolton & Imaizumi, 1996). STOCs recorded in mouse colonic smooth muscle cells were shown to be generated by the opening of both SK channels and BK channels in response to transient increases in submembrane Ca^{2+} (Kong, Koh & Sanders, 2000) suggesting that these channels are co-localized in the cell membrane.

Although the activity of BK channels is regulated primarily by the intracellular [Ca^{2+}], a study on vascular smooth muscle cells showed that BK channels can be directly activated by NO (Bolotina, Najibi, Palacino, Pagano & Cohen, 1994), leading to vasodilation. In GI smooth muscle the action of NO on BK channels is associated with an increase in their open probability, although the mechanism by which this occurs is not clear. A recent study on BK channels in excised inside-out patches from smooth muscle cells of the guinea-pig teania coli concluded that redox modulation and nitrothiosylation of cysteine groups on the α subunit of the BK channels alone alters channel gating but is not mediated by the generation of cGMP (Lang, Harvey, McPhee & Klemm, 2000). However, in cell-attached patches, the open probability of BK channels comprising the α subunit cloned from canine colonic smooth muscle, and expressed in HEK293 cells, is increased upon application of NO donors such as sodium nitroprusside to the bathing solution (Fukao *et al.*, 1999). Direct application of an active subunit of cGMP-dependent protein kinase (PKG), in the presence of cGMP and ATP, to the cytoplasmic surface of inside-out patches containing these channels also increased their open probability, indicating that PKG-dependent phosphorylation opens BK channels cloned from colonic smooth muscle. Moreover, point mutation of a one serine molecule on a C-terminal PKG-consensus sequence abolished the effect of NO (Fukao *et al.*, 1999). Therefore, it is possible that the specific redox form of NO which is generated by different donors determines whether BK channels are activated directly by NO or through PKG-dependent phosphorylation of the channel.

The physiological role of NO-modulation of BK channels in smooth muscle is unclear, because the

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NO-dependent IJPs that have been recorded in GI smooth muscle are insensitive to low concentrations of TEA which block BK channels (Goyal & He, 1998). NO, however, may inhibit muscle contractions by decreasing the amplitude of action potentials and shortening their duration; thus restricting Ca^{2+} entry. BK channels are also targets for modulation by inflammatory agents in the gut and their activity was shown to be up-regulated in smooth muscle cells of the canine colon following inflammation of the mucosa (Lu *et al.*, 1999).

IK and SK channels

Classification of Ca^{2+}-activated K^+ channels other than BK (unitary conductances less than ~180 pS) has been somewhat arbitrary as a result of the wide variation in unitary conductances. A number of SK and/or IK channels with conductances ranging from <10 to 40 pS have been cloned from tissues of the brain (Kohler *et al.*, 1996), pancreas (Ishii, Silvia, Hirschberg, Bond & Adelman, 1997b) and placenta (Joiner, Wang, Tang & Kaczmarek, 1997). Not all the cloned SK channels, however, are blocked by nanomolar concentrations of apamin (Kohler *et al.*, 1996) which has been used as a probe to test for the expression of SK channels, both in binding studies and as a blocker of ionic currents. Although all SK channels are resistant to millimolar concentrations of external TEA, the isoforms expressed in placental (Joiner *et al.*, 1997) and pancreatic tissue (Ishii *et al.*, 1997b) are inhibited by ChTX. D-Tubocurarine (d-TC), the nicotinic receptor blocker, is also a very effective blocker of the apamin-sensitive isoforms of cloned SK channels (Kohler *et al.*, 1996) and competes with apamin for the same binding site (Ishii *et al.*, 1997a). The block of SK channels by d-TC is an important consideration if d-TC is to be used for the purpose of blocking nicotinic receptors in myenteric ganglia. This is because enteric neuronal inhibitory motor reflexes that involve activation of apamin-sensitive IJPs on the muscle (Spencer, Bywater & Taylor, 1998) may wrongly be interpreted as involving nicotinic ganglionic neurotransmission.

Apart from having 10-fold lower unitary conductances, SK and IK channels differ from BK channels by having no apparent voltage-dependence despite the presence of three positively charged amino acids in the S4 region of the channel (Ishii *et al.*, 1997b). This property conforms to the properties of classic SK channels which are gated by intracellular Ca^{2+} alone (Blatz & Magleby, 1987; Latorre, Oberhauser, Labarca & Alvarez, 1989). The nature of this gating has now been elucidated and involves transduction of changes in Ca_i^{2+} by calmodulin which is constitutively bound to the C-terminal region of SK channels (Xia *et al.*, 1998). Binding of Ca^{2+} to calmodulin is thought to induce a conformational change in the SK channel allowing it to conduct K^+.

Cloned SK channels have EC_{50} values < 1 μM Ca_i^{2+} and Hill coefficients of 4–5 suggesting cooperative gating (Kohler *et al.*, 1996). In smooth muscle cells of the mouse colon, Koh *et al.* (1997) reported that the 5.3-pS SK_{Ca} channels had an EC_{50} of 490 nM. In smooth muscle cells of the mouse ileum, although the ~10-pS SK_{Ca} channels were sensitive to Ca_i^{2+} and blocked by internal Cs^+ (see Fig. 3) the EC_{50} for Ca^{2+} measured from macroscopic currents in excised patches was estimated to be 1.2 μM and the Hill coefficient averaged 1.7. This slope is lower than that reported for the cloned channels or for native channels in neuroblastoma cells (Leinders & Vijverberg, 1992) suggesting that the SK channels in smooth muscle may be less sensitive to Ca_i^{2+} than in other tissues.

The intermediate conductance 90- and 150-pS channels in CM cells of the canine colon (Koh *et al.*, 1996) and the 40-pS K_v channels in the mouse ileum are also sensitive to Ca_i^{2+} (Vogalis *et al.*, 1998b). Although the molecular counterparts of these channels have yet to be identified, it seems unlikely that they are encoded by isoforms of the cloned SK channels and may be more closely related to K_v channels whose activities are increased by cytoplasmic Ca^{2+} through second messenger pathways. Recently, Joiner *et al.* (1998) reported that co-expression of cloned BK (*slo*) channels and a novel K^+ channel which is inhibited by Ca_i^{2+} (*Slack*) produces Ca^{2+}-activated K^+-channels that have conductances in the range of 60 pS. Therefore, these heteromeric channels may encode some types of non-BK voltage-gated and Ca^{2+}-dependent IK channels in GI smooth muscle.

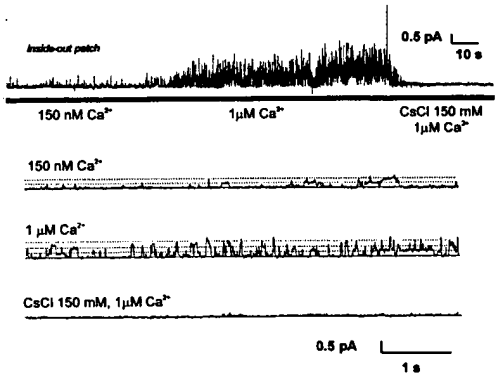

Figure 3 Inside-out patch recording from a smooth muscle cell of the mouse ileum. Upper trace, An increase in $[Ca^{2+}]$ on the cytoplasmic side of the patch increased the opening of SK_{Ca} channels. Substitution of K^+ with Cs^+ blocked these channels. Lower traces, Expanded portions of the trace shown in panel A. TEA 5 mM was added to the pipette solution to block BK channels and the holding potential was 0 mV to inactivate K_v channels (F. Vogalis, unpublished data).

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Functional role of SK channels

IJPs in many GI smooth muscle preparations are blocked or markedly inhibited by apamin (Niel, Bywater & Taylor, 1983; Shuba & Vladimirova, 1980). These events are produced by the action of inhibitory transmitter(s) acting on receptors on the smooth muscle cells and lead to hyperpolarization of the membrane potential and inhibition of the opening of Ca_v channels. ATP is candidate neurotransmitter which is released from inhibitory motor nerves to produce these apamin-sensitive IJPs and bath-applied ATP has been shown to hyperpolarize various GI smooth muscles including the internal anal sphincter (Rae & Muir, 1996) and guinea-pig colon (Zagorodnyuk & Maggi, 1998) by activating apamin-sensitive K^+ conductances. In addition, in smooth muscle cells of the mouse ileum (Vogalis & Goyal, 1997) and colon (Koh *et al.*, 1997), purinoceptor agonists have been shown to stimulate the opening of apamin-sensitive SK channels.

The depolarization produced by apamin in GI smooth muscle such as CM of the guinea-pig ileum (Niel *et al.*, 1983) suggests that SK channels may be active at the resting potential. In the CM of the guinea-pig stomach, however, the RMP was not altered by apamin which nevertheless increased the duration and amplitude of slow waves and increased spiking in the muscle (Suzuki *et al.*, 1993). This discrepancy in the contribution of apamin-sensitive SK channels to the resting potential may be related to tissue-specific expression of apamin-sensitive vs. apamin-resistant isoforms of SK channels containing different stoichiometries of apamin-sensitive and apamin-resistant subunits. Interestingly, the inhibitory action of apamin on the CM of the guinea-pig stomach was more pronounced when $[Ca^{2+}]$ in the bathing solution was increased to 5 from 1.5 mM, a manoeuvre that would be expected to lead to higher levels of cytoplasmic Ca^{2+} (Williams, Fogarty, Tsien & Fay, 1985). Given that SK channels are gated by Ca^{2+}-calmodulin, it is possible that at low basal levels of internal Ca^{2+} the binding sites on calmodulin for Ca^{2+} may be occupied by Mg^{2+} in the cytoplasm, which is at a concentration of ~ 1 mM, thus maintaining SK channels in a closed state. SK channels may then open only after cytoplasmic or submembrane $[Ca^{2+}]$ exceeds a critical level, enabling Ca^{2+} to displace Mg^{2+} from calmodulin (see Malmendal *et al.*, 1998).

Other K^+ channels in GI smooth muscle

Inwardly rectifying K_{ATP} channels

Intracellular ATP can directly gate, or shut, a class of K^+ channels that are found in many types of excitable cells (Edwards & Weston, 1995). Therefore, the open probability of these K_{ATP} channels is directly linked to the metabolic activity of the cell: opening when the ADP/ATP ratio in the cytoplasm increases; and closing when the level of ATP approaches the millimolar range (Edwards & Weston, 1995). Unlike K_v channels, the pore-forming subunits that constitute K_{ATP} channels each contains only two membrane spanning regions (Ashcroft & Gribble, 1998). A number of studies have confirmed the presence of K_{ATP} channels in GI smooth muscle. Outward currents generated by agonists of K_{ATP} channels have been recorded in cells of the muscularis mucosa of the rabbit oesophagus and are blocked by glibenclamide and by muscarinic receptor stimulation (Hatakeyama, Wang, Goyal & Akbarali, 1995). In smooth muscle of the guinea-pig gallbladder, the hyperpolarization elicited by CGRP is mediated by the opening of K_{ATP} channels through the activation of cAMP-dependent protein kinase (Zhang, Bonev, Mawe & Nelson, 1994). A recent study of Koh *et al.* (1998) reported that K_{ATP} channel agonists activated a 27-pS K^+ channels in smooth muscle cells of the mouse ileum that was responsible for the hyperpolarization recorded in intact muscle strips elicited by lemakalim. RT-PCR analysis on this tissue identified transcripts for $K_{ir}6.2$ and for the SUR2A subunit that binds glibenclamide (Koh *et al.*, 1998). It should be mentioned that K_{ATP} channel openers have been shown in smooth muscle cells of the LM layer of the guinea-pig ileum to induce a voltage-independent outward current while at the same time inhibiting a delayed-rectifier-type I_{K_v} (McHugh & Beech, 1995) suggesting that cromakalim may also alter the voltage dependence/gating of K_v channels.

In muscle strips of guinea-pig taenia caeci, cromakalim hyperpolarized the muscle, an action that was blocked by glibenclamide (Den Hertog, Van den Akker & Nelemans, 1989) suggesting that K_{ATP} channels are expressed in this tissue. Similarly, in the CM of the canine colon, pinacidil, an opener of K_{ATP} channels, hyperpolarized canine colonic CM strips (Post, Stevens, Sanders & Hume, 1991). The relaxation produced in muscle strips of mouse gastric fundus by field stimulation of enteric motor nerves, however, was unaffected by glibenclamide. This suggests that the inhibitory (non-cholinergic non-adrenergic, NANC) neuromuscular transmission in GI smooth muscle does not involve the opening of K_{ATP} channels on the muscle (Selemidis & Cocks, 2000) which supports similar results of contractility studies in the rat ileum (Franck, Puschmann, Schusdziarra & Allescher, 1994). Although K_{ATP} channel agonists cause both hyperpolarization and relaxation in GI smooth muscle, these agents also relax smooth muscle strips pre-contracted with depolarizing concentrations (20–30 mM) of external K^+ that directly act on the muscle (McPherson & Angus,

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1990; Richardson, Alibhai & Huizinga, 1992; Sun & Benishin, 1994). This suggests that the membrane hyperpolarization elicited by these K_{ATP}-channel agonists may not be the cause of the relaxation produced by these agents. However, reduction in tone may be related to the ability of the cromakalim, but not lemakalim, to directly inhibit L-type Ca^{2+} channels in smooth muscle (Post *et al.*, 1991).

K^+ channels inhibited by muscarinic agonists

M-current channels were first described in bullfrog sympathetic neurones and were defined as a voltage-dependent K^+ current that was active at rest and was inhibited by muscarinic agonists (Marrion, Smart, Marsh & Brown, 1989). M-currents are widely expressed in neuronal preparations and the conductance of M-current channels is of the order of ~ 7 pS (Selyanko & Sim, 1998). In mammalian GI smooth muscle, however, there is little evidence for an M-current, *per se*, although muscarinic receptor stimulation may inhibit BK channel currents (Cole, Carl & Sanders, 1989) and also $I_{K_{yslow}}$ in CM cells from the guinea-pig ileum (F. Vogalis, unpublished observations). In mammalian GI smooth muscle, evidence for inhibition of a voltage-dependent, non-inactivating K^+ current which is significantly activated at resting potentials (from -50 to -60 mV) and which is inhibited by muscarinic agonists (i.e. M-current) is not available. In toad gastric smooth muscle cells, however, a voltage-dependent K^+ channel current which is inhibited by muscarinic agonists is active at resting potentials (Sims, Singer & Walsh, 1985) suggesting that expression of M-type K^+ channels may be species-specific.

Concluding remarks on K^+ channels and GI motility

The diversity of K^+ channels expressed in GI smooth muscle reflects the fine control that K^+ channels exercise in the excitability of smooth muscle. The motility of the GI tract is intricately controlled by the activities of extrinsic and enteric nerves that release excitatory and inhibitory transmitters onto the syncytium. Smooth muscle provides the mechanical forces to process food, and each part of the GI tract is specialized to perform specific functions. These functions may depend ultimately on the electro-mechanical properties of the smooth muscle in the different regions, which may express different complements of ion channels. By knowing the identity of these channels, in particular the types of K^+ channels expressed, it may be possible to specifically control the activity of different regions of the GI tract, to either increase or decrease transit time and perhaps alter feeding behaviour by affecting gastric emptying.

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